PROGRAMMABLE LIFESPAN TIMER

Lily Lin, Rebecca Poch, Nolana Wong, Jae Jang

iGEM 2014

Purpose

- Create a "lifespan timer" for cells used in production. In case they leave the lab environment, they will die after a set amount of time, preventing the release of unwanted chemicals into the environment, or mating with wild type organisms.

The Function of Telomeres

When cells undergo cell division, their telomeres shorten. When the telomeres have reached a certain length, the cell will reach senescence and die.

Normally, the enzyme telomerase prevents this by adding nucleotides to the telomere ends.
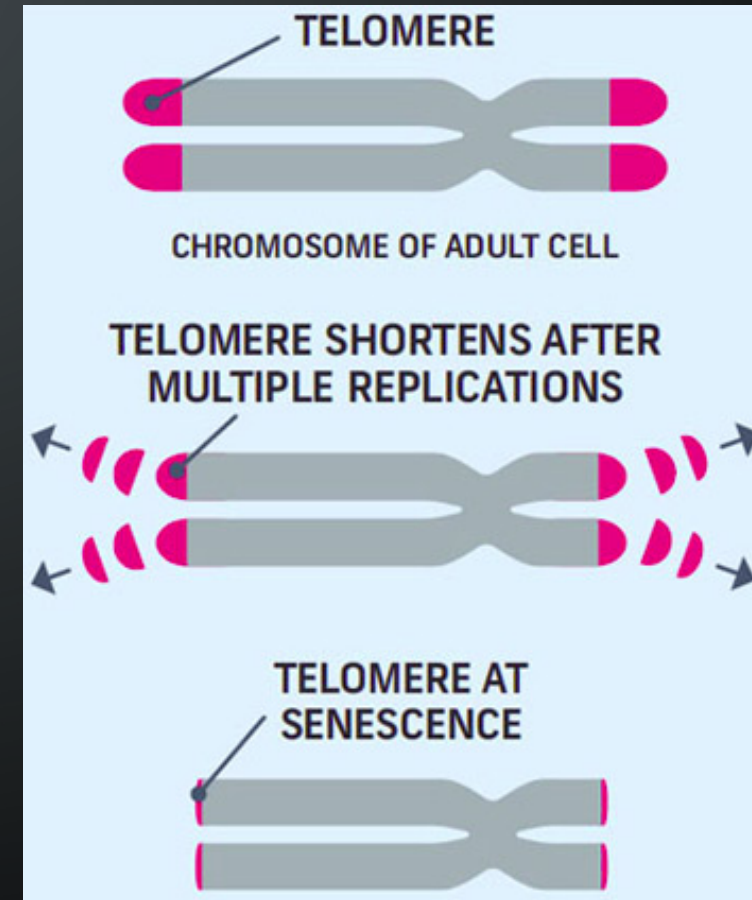
Our Objective

We are using telomere lengths to control the lifespans of organisms that can be used in production, which can be achieved by knocking out certain genes within the genome.

We are knocking out EST1, RAD52, MAK31, and VPS75 using a gene deletion protocol. This protocol knocks in a selectable marker (TRP and LEU) in place of the deleted gene.
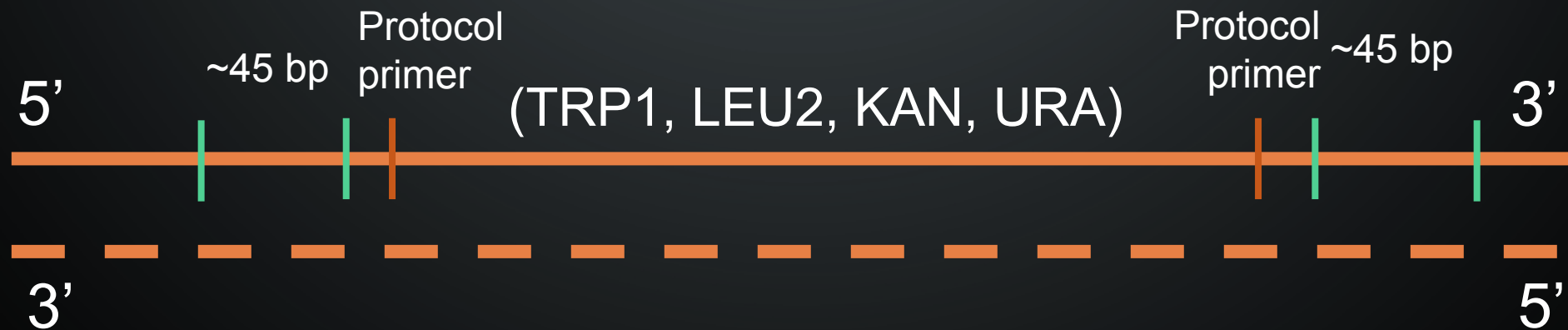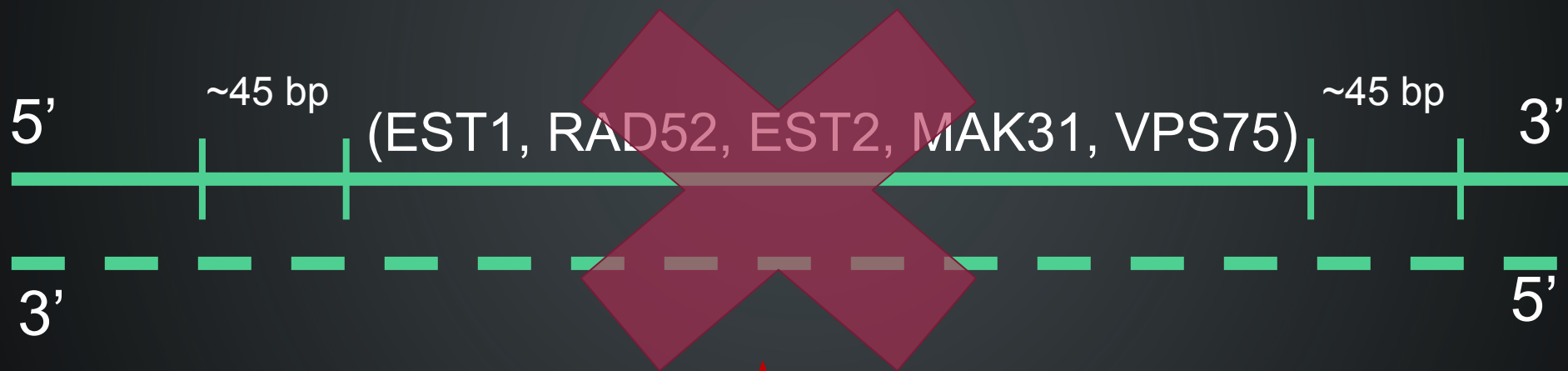
Genes to Knock Out

EST1
  - Telomerase gene

RAD52
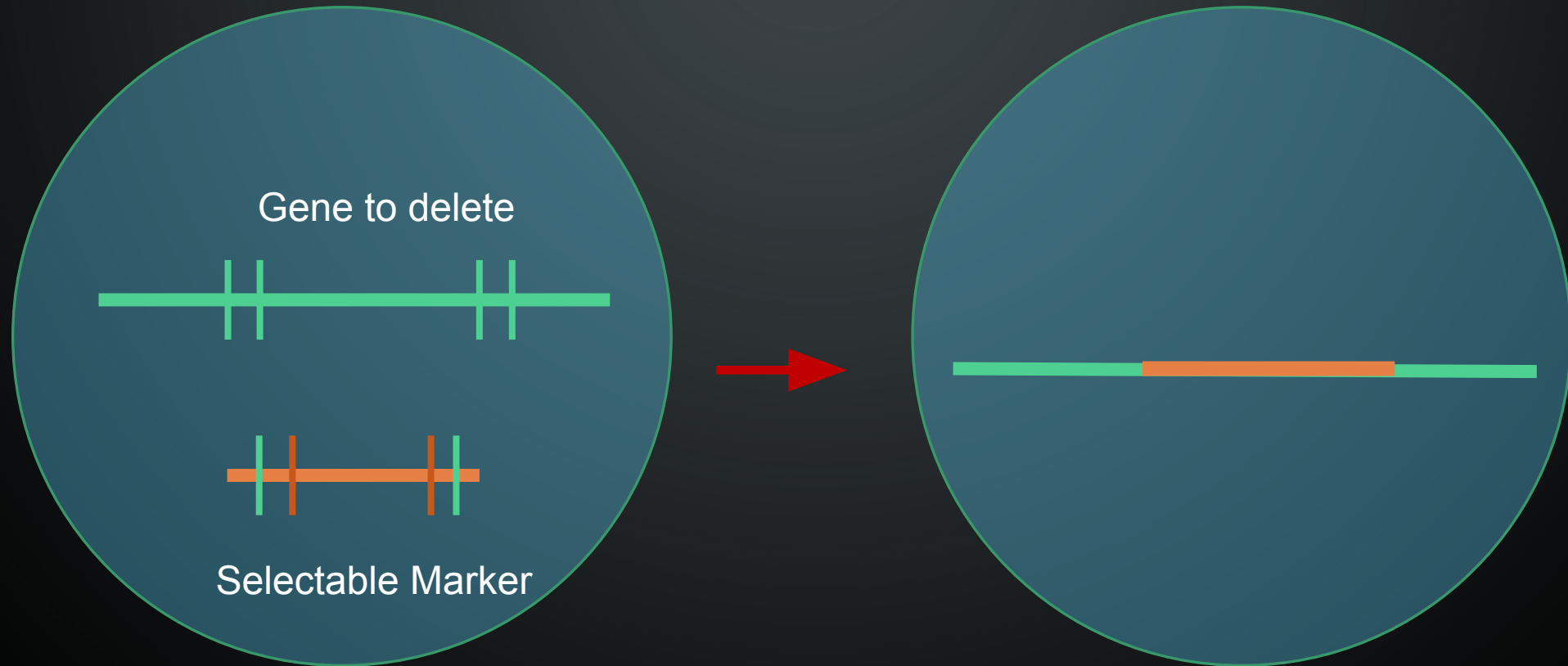 - Alternate telomere recovery pathway

MAK31
  - Knocking out will lengthen telomeres

VPS75
  - Knocking out will shorten telomeres

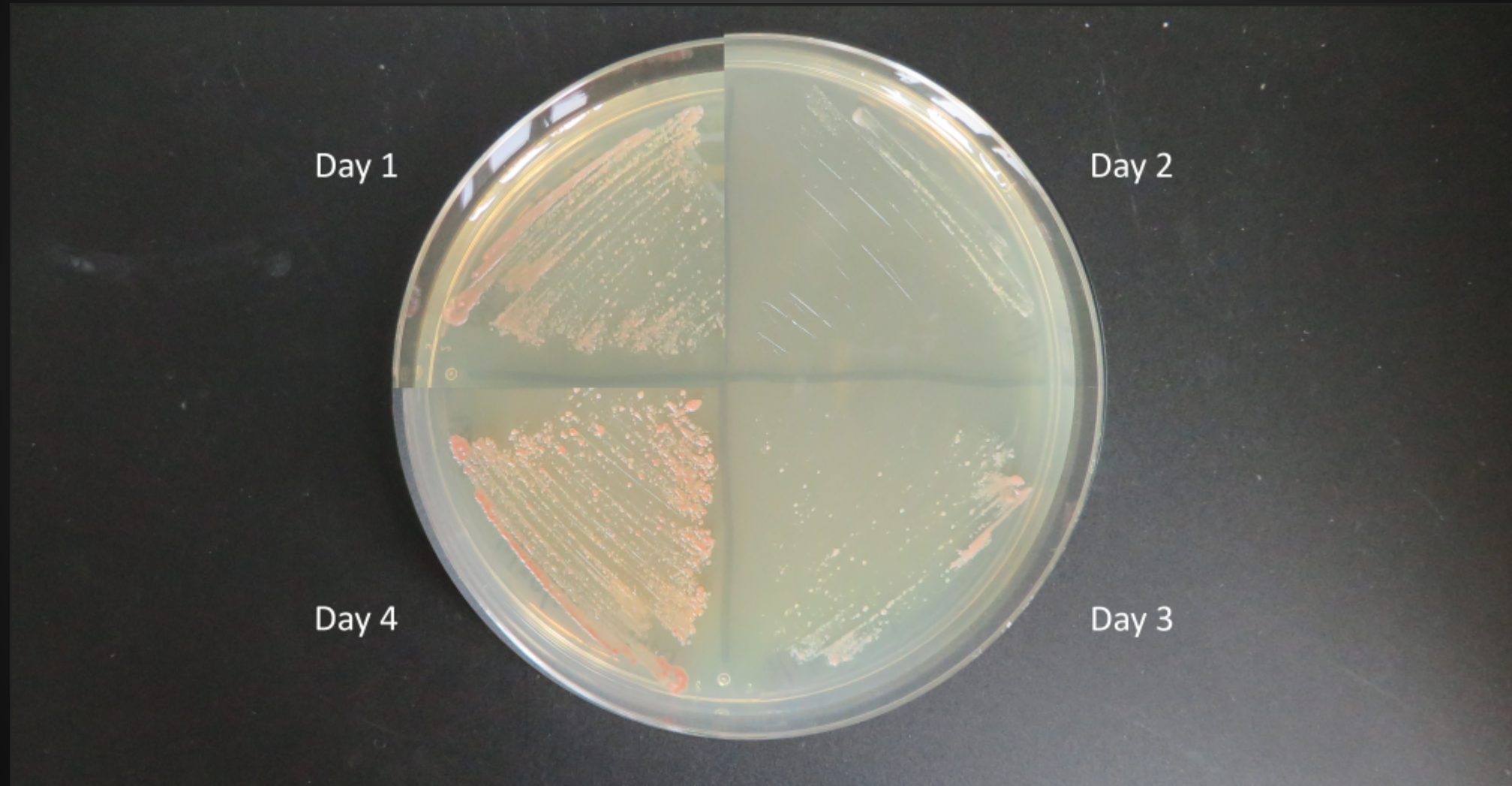KNOCKOUT CASSETTE

TRANSFORM YEAST


Gene to delete

Selectable Marker

What We've Done

- Working with yeast strains (W303A/W303alpha) to test the effect of gene knockouts on yeast lifespan.
- Monitored the growth of yeast with EST2::URA as a control.
- Making knockouts of MAK31 and VPS75 in conjunction with EST1 to compare yeast growth.

Senescence in EST::URA Knockouts

Homologous Recombination

- When two sister chromatids exchange corresponding parts of their DNA

- RAD52 allows for homologous recombination in yeast strains with telomerase genes knocked out, which allows the yeast to recover from senescence
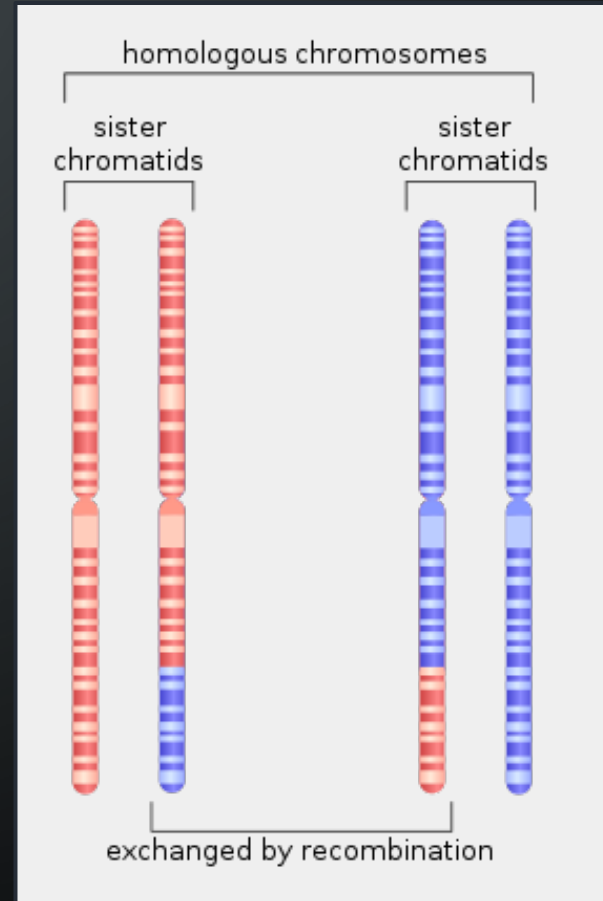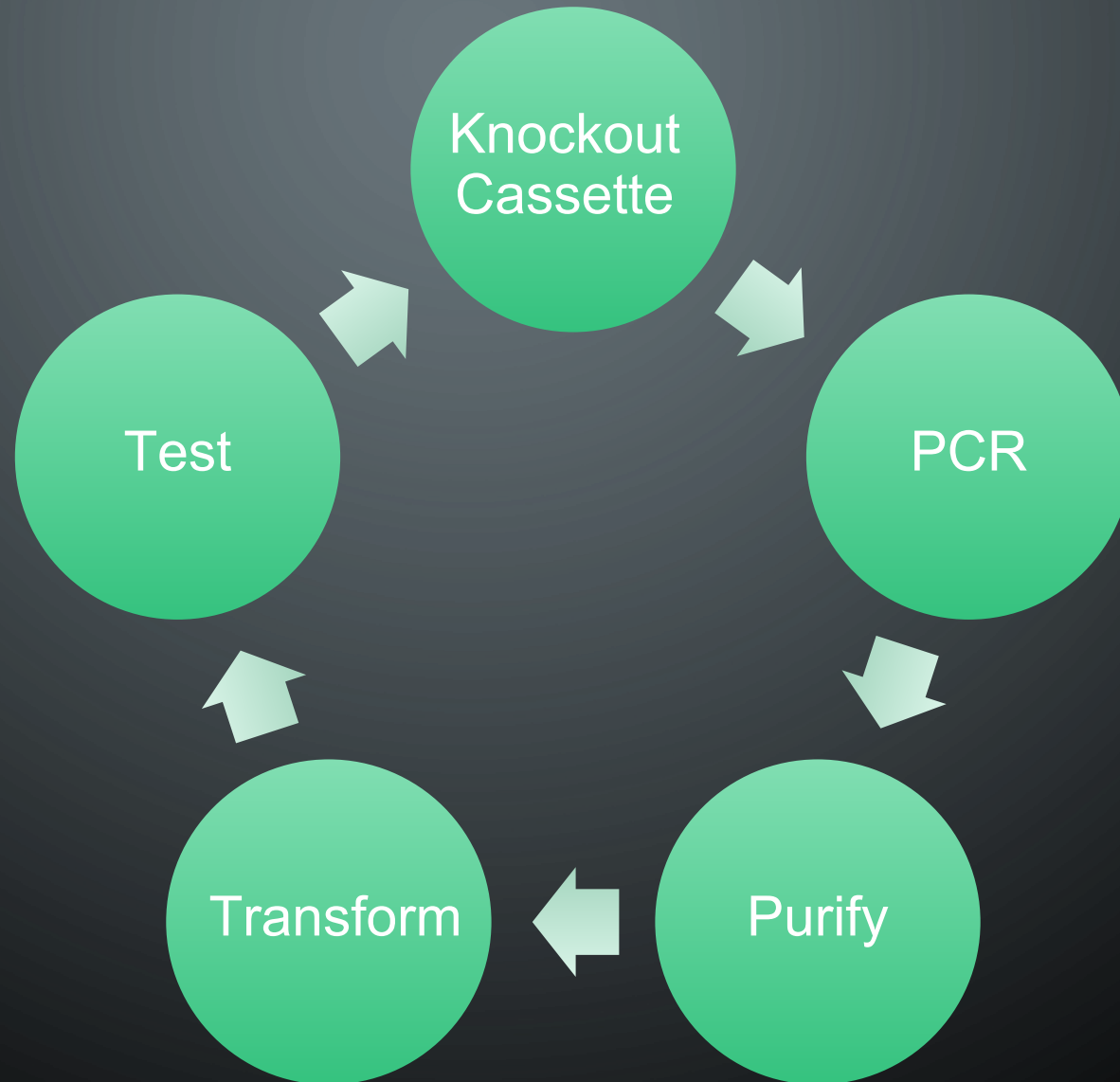
Growth Curve

- Graphs that compare number of colonies over time.

- Knocking out telomerase genes in yeast with shorter, normal-length, and longer telomeres will generate growth curves that show shifts in when yeast cells start dying off.
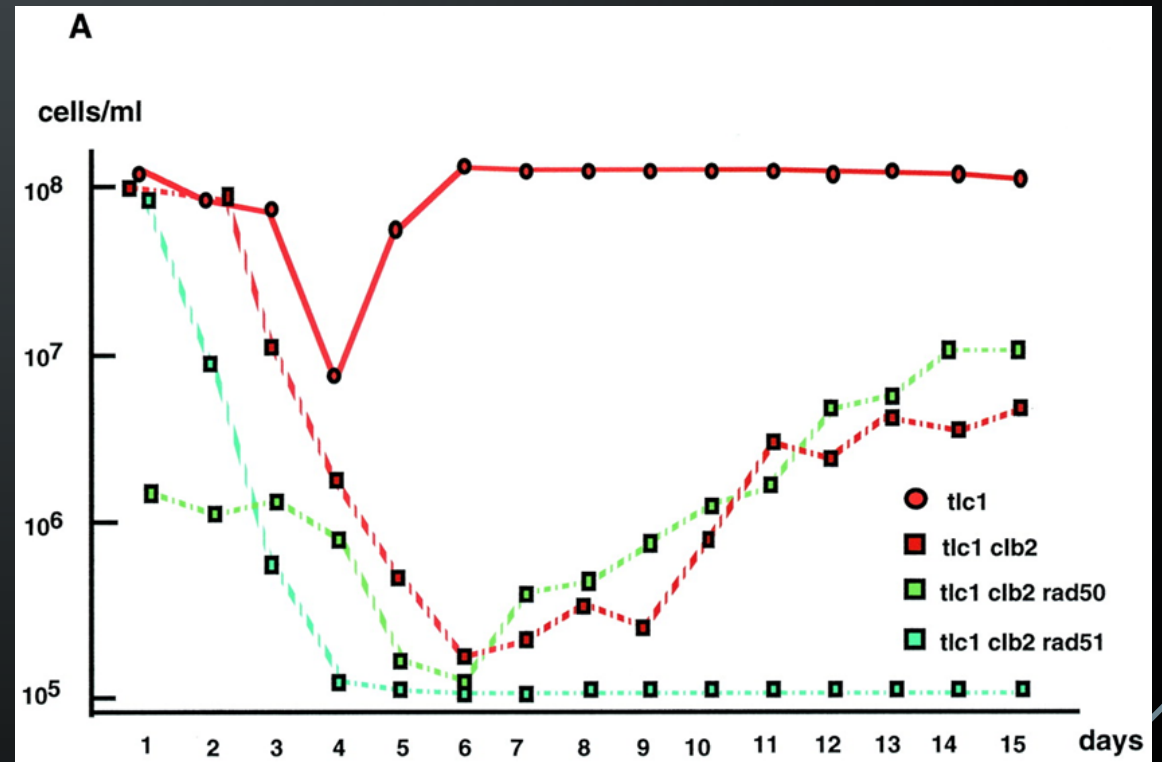
Programmable Life Switch

What's Next?

Assuming that the cassettes are successful, we need to knock out the mating genes of the haploid yeast strains to prevent mating with wild-type yeast.

After working with yeast, we hope to transfer the system to other organisms such as E. coli.

Questions?